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A BRIEF RÉSUMÉ OF VERNES' METHOD FOR SERUM DIAGNOSIS OF SYPHILIS.[1]

Biologic and Physical—Chemical Phenomena of the Serum Reaction.

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(1) Vernes ascertained that a metallic or an organic colloidal suspension in distilled water, or water containing certain electrolytes in varying concentrations, is flocculated when mixed with human-blood serum. This flocculation occurs with a definite rhythm which varies according to the quantities of serum added. There is a zone of flocculation with certain quantities of human serum alternated with a zone of nonflocculation, and one obtains a characteristic picture of interposed zones of flocculation and nonflocculation. This phenomenon ceases to take place when the quantities of serum become too minute to produce any effect upon the equilibrium of the colloidal suspension. Vernes observed this phenomenon first with a colloidal iron suspension of certain concentrations and then with several other inorganic colloids as well as with those of an organic nature.

When these colloidal suspensions were mixed with normal human serum, the flocculation did not occur in the same quantities as it did when the suspensions were mixed with the serum from a syphilitic person; that is, the flocculation zone with normal serum did not occur with the same quantities as it did with syphilitic serum.

Utilizing this fact, Vernes proposed a method by which a normal serum could be differentiated from that of a syphilitic. His method consisted in employing such quantities of serum as those which produce a flocculation of colloidal suspension with syphilitic serum only. By using certain quantities any human serum can, of course, induce the same phenomenon; but this is avoided by a careful selection of quantities within which no nonsyphilitic serum can cause a flocculation.

(2) Vernes found after further experiments that an extract from the heart muscles of the horse, as obtained by a thorough extraction of substances that can be dissolved out by means of perchloride of ethyl and alcohol from thoroughly desiccated powder of the muscle (using Soxhlet apparatus at 41°–45° C. under reduced pressure), gives a colloidal suspension more stable than colloidal iron when mixed with saline water. It not only yields a finer suspension, but it enables a differentiation of a syphilitic from a normal serum with greater definition. The substances extracted with perchloride of ethyl and then alcohol, from the dried powder of horse's heart muscle, are called "perethynol," from *per* (chloride of) *ethyl,* and *alcohol.* This mass of substances contains various lipoids.

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[1] From L'Institut Prophylactique, Paris.

(3) In a reaction dealing with a colloidal suspension it is of the utmost importance to produce a constant suspension each time, capable of being standardized, to insure a uniform degree of molecular dispersion; otherwise the results obtained could not be compared with each other. The flocculation zone naturally shifts toward one direction or the other, according to the degree of dispersion. For this reason it was prerequisite that a scale of opacity or opalescence should be prepared for standardizing the perethynol suspension on each occasion when one was made.

This scale was devised by Vernes, who utilizes a mixture of tinctures of benzoin and quillaja with certain quantities of glycerinated water, which mixture is fairly stable if kept under certain conditions. Vernes prepared several grades of opalescence, and recommends that one of them, which he designated as grade 3, be used as the standard for the perethynol suspension to be used in the reaction. As a matter of fact a 1/40 dilution in saline solution gives the desired opalescence; if slightly more or less, it is easily adjusted to the exact scale. Particular stress is laid upon the importance of mixing the perethynol with saline solution by dropping the latter from a burette to the former under constant agitation. A special set of apparatus (agitator, burette, etc.) is employed by Vernes.

(4) Vernes conceived the idea of translating the flocculation phenomenon into a color reaction which would be easier to measure. His idea was to find a substance which would indicate the degree of changes in the syphilitic serum by losing one of its activities while counteracting the flocculating effect of the serum upon the colloidal suspension of perethynol.

According to Vernes, a fresh serum of the pig fulfills this purpose as it acts as an antiflocculent toward the syphilitic serum and at the same time loses some of its hemolytic property upon sheep's red blood corpuscles. When mixed with a normal serum, the antiflocculent property of the pig's serum is not used up; hence there is no loss of its hemolytic activity.

According to Vernes, the degree of loss in the hemolytic power of pig's serum when mixed with the syphilitic serum and perethynol suspension corresponds with the degree of the specific changes in the human serum in question. From this he proceeded to set up an accurate determination of the change in human serum, due to syphilis, by introducing a colorimetric scale consisting of eight degrees of intensity. His scale is a series of artificial tints made to approximate those of various dilutions of laked red corpuscles. The grades are divided between 0 (absence of hemolysis) and 8 (complete hemolysis), with intermediary tints. The color scale of Vernes contains acid fuchsin, picric acid, formol, etc., and is comparatively stable in the dark. It may be seen that once a color scale is provided, it is easy to prepare a uniform suspension of sheep's corpuscles on each occasion by taking that concentration which will give the color scale 8 when laked in a definite (constant) volume of fluid.

(5) Pig's serum is collected at a slaughterhouse and is used within 24 hours; it should be free from red corpuscles and hemoglobin tint. The quantity used is constant in volume and hemolytic power (against washed sheep's red corpuscles). As a rule, a titration carried out in two sets of several tubes (one set with undiluted and the other with diluted serum) shows whether the sample is sufficiently strong or not. The majority of pig's serums contain enough amboceptor and complement.

(6) Sheep's corpuscles should be washed with saline solution and made up to the desired suspension. The patient's serum is heated to 55° C. for 20 minutes just before being used in the test. It must be free from hemoglobin or corpuscles (centrifugalization is employed).

(7) The following is the method of procedure: Every reagent must be contained in a constant volume (0.8 cc.) of saline solution, except the patient's serum (0.2 cc.). Spinal fluid is used unheated in quantity of 1.6 cc. The tubes (11 mm. inner diameter) receive the patient's serum, perethynol suspension, and pig's serum, and are then placed in a thermostat (37° C.) for 75 minutes. At the end of incubation, sheep's corpuscles are added to all and these are once more incubated at 37° for hemolysis which is usually complete in from 28 to 30 minutes. Those tubes which show partial hemolysis are at once centrifugalized, and the intensity of tint in the supernatant fluid is read with the aid of the artificial colorimeter. Appropriate controls are provided.

(8) A chart system of recording the colorimetric determinations on different occasions is kept for every patient and is called by Vernes, "syphilimetrie." The chart is marked with the grades of tints from 0 to 8. It is claimed by him that the absence of the reaction beyond eight months after the treatment ceased indicates a permanent cure of syphilis

(9) Incidental to this method, Vernes devised a rheometer (for measurement of a definite amount of serum) and an aspiropipeur (a syringe which aspirates a liquid from a container and forces it out by a piston into the test tubes) for distributing a given quantity of reagent to a large number of test tubes in rapid succession.

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